Supplemental information

Table S1. The expression levels of several maturation genes and *Syt* genes in purified *Mip*^{eGFP} β cells, related to Figure 2.

	Sample FPKM value								
Gene Name	**P0**	**P0**	**P0**	**P12**	**P12**	**P12**	**P60**	**P60**	**P60**
Known genes in maturation									
MafA	52.29	47.23	32.18	73.83	75.06	74.74	365.52	340.77	296.19
Neurod1	52.32	49.42	62.42	45.58	49.45	49.68	101.62	85.62	81.20
Ucn3	26.82	22.94	12.27	86.28	109.24	88.24	384.48	404.48	313.66
***Syt* gene**									
Syt1	0.66	0.83	0.88	0.27	0.59	1.26	0.30	0.09	0.00
Syt2	0.88	0.94	0.67	1.49	1.68	1.72	2.39	2.10	2.72
Syt3	3.50	3.76	2.32	2.22	3.08	0.00	11.17	9.04	10.56
Syt4	3.29	3.11	1.97	8.10	9.78	9.42	56.12	53.66	55.48
Syt5	8.94	9.71	12.99	14.21	15.70	15.63	44.38	36.68	41.93
Syt6	0.13	0.16	0.10	0.07	0.08	0.07	0.09	0.04	0.05
Syt7	35.04	33.47	43.25	24.97	31.83	28.48	42.91	37.14	37.46
Syt8	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Syt9	4.23	4.56	4.75	5.62	7.54	6.75	3.14	4.18	3.00
Syt10	0.11	0.07	0.00	0.12	0.20	0.11	21.81	27.12	18.66
Syt11	7.82	7.41	6.20	2.95	3.87	4.40	8.51	8.76	7.15
Syt12	0.27	0.30	0.56	0.35	0.28	0.23	0.12	0.05	0.19
Syt13	136.23	128.06	132.83	106.44	121.94	103.30	349.57	339.20	308.93
Syt14	10.95	10.14	9.20	9.73	12.56	12.38	15.10	16.57	16.83
Syt15	0.35	0.87	0.13	0.67	0.83	1.58	0.08	0.00	0.00
Syt16	2.47	2.83	3.08	1.23	1.30	1.75	0.12	0.06	0.47
Syt17	2.64	2.00	3.59	0.78	0.57	0.99	0.07	0.00	0.14

Shown are normalized RNA-seq data from FACS-purified β cells at three stages (fragments per kb mRNA per million read, FPKM). Triplicate samples were examined at each particular stage.

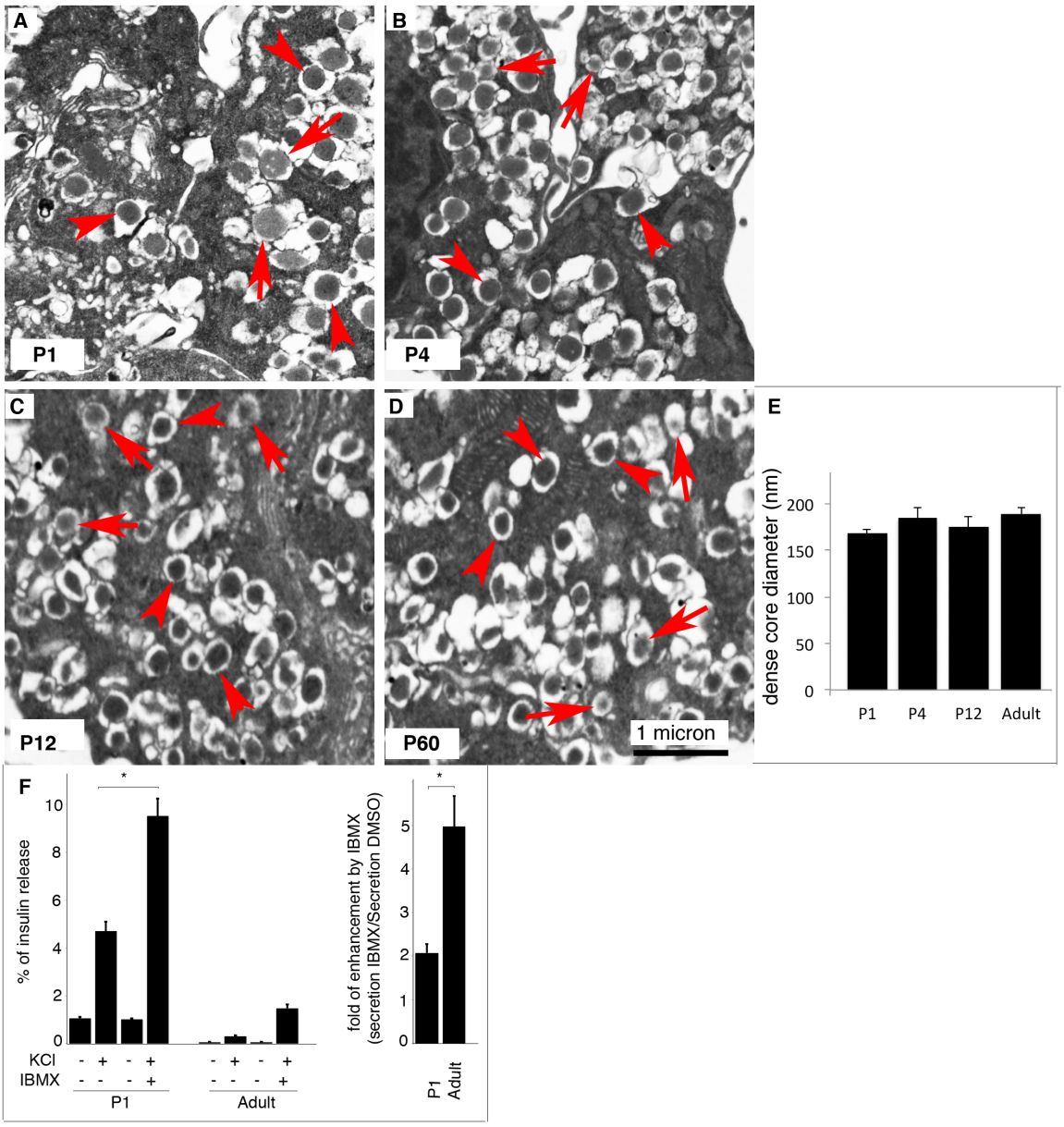
Table 2S. Oligos used for the studies, related to Figure 2-7 genotyping and real-time RT-PCR.

Gene	oligos	expected size/name
mSyt3 (RT-PCR)	gaacactgggcagagatgct	
	tggcttcgaggagatgagtt	
mSyt4 (RT-PCR)	atggctcctatcaccaccag	
	agcagatccaggcaaagaga	
mSyt5 (RT-PCR)	tcaccgtcattgtcttggaa	
	aagctgaaggcctcgttgta	
mSyt7 (RT-PCR)	gaggtgtccatccctctgaa	
	gcagaggggttgtagcagag	
mSyt9 (RT-PCR)	cactggcactctctgatgga	
	catggttgtccgactgtgtc	
mSyt11 (RT-PCR)	agctgaccagggacatcatc	
	atccatcttcggcaagtgtc	
mSyt13 (RT-PCR)	ggggaggtccttctgtcaat	
	gcttctgagcctggtgtttc	
mSyt14 (RT-PCR)	acagcagtccttagccctga	
	tggctctggatcaaaaggtt	
RipmCherry (genotyping)	ctgcagaagcg ggcattgtgga	280 bp
	ggtgcttcacgtaggccttgga	
Rip-rTTA (genotyping)	gtgaagtgggtccgcgtacag	400 bp
	gtactcgtcaattccaagggcatcg	
Syt4 null (genotyping)	cacttccctcacgtcagaggag	WT: 300 bp Mut: 250 bp
	gcaaggagagctcttggatgtg	
	aaccacactgctcgacattggg	
TetO-Syt4 (genotyping)	aagaagcacagagtgaagaccag	350 bp
	ggatctgaaagtccagacacatc	
hSyt4 (RT-PCR)	ttcaggacggggtgagttac	
	tttggcatggtacaggttca	
hPDX1 (RT-PCR)	tactggattggcgttgtttgtggc	
	agggagccttccaatgtgtatggt	
hGLUT1 (RT-PCR)	ggacaggctcaaagaggttatg	
	aggaggtgggtggagttaat	
hPRE-INSULIN (RT-PCR)	gtgaaccaacacctgtgcgg	
	aggggcagcaatgggcagtt	
hINSULIN (RT-PCR)	agaggccatcaagcagatcactgt	
	aggtgttggttcacaaaggctg	
hGCK (RT-PCR)	ccttcttcaggtcctcctcc	

	gatggatgtcacaaggagcc	
hMAFB (RT-PCR)	accttggctaaggcgagagtag	
	cttcagcctggagagaagttactc	
hMAFA (RT-PCR)	tgagcggagaacggtgatttctaagg	
	ggaacggagaaccacgttcaacgta	
hNEUROD (RT-PCR)	attgcaccagcccttcctttgatg	
	tcgctgcaggatagtgcatggtaa	
hNKX6.1 (RT-PCR)	attcgttggggatgacagag	
	cgagtcctgcttcttcttgg	
hSLC30A8 (RT-PCR)	atcgaagcctccctctaagc	
	cacatgccaggtacactagca	
hGAPDH (RT-PCR)	aatcccatcaccatcttcca	
	tggactccacgacgtactca	
Myt1LF (genotyping)	gtatggggaaactgctgaatgaa	P1
	gaggaggcaacataactgaaga	P2
	gcatccagacagactgcggtga	P3 (WT: 476nt, F: 550nt, null: 350nt)
St18F (genotyping)	ggagcatgttcaccttctgg	P4
	tgagactgagactacttgttagc	P5
	gctttctgggtttcatttctg	P6 (WT: 408nt, F: 480nt, null: 380nt)

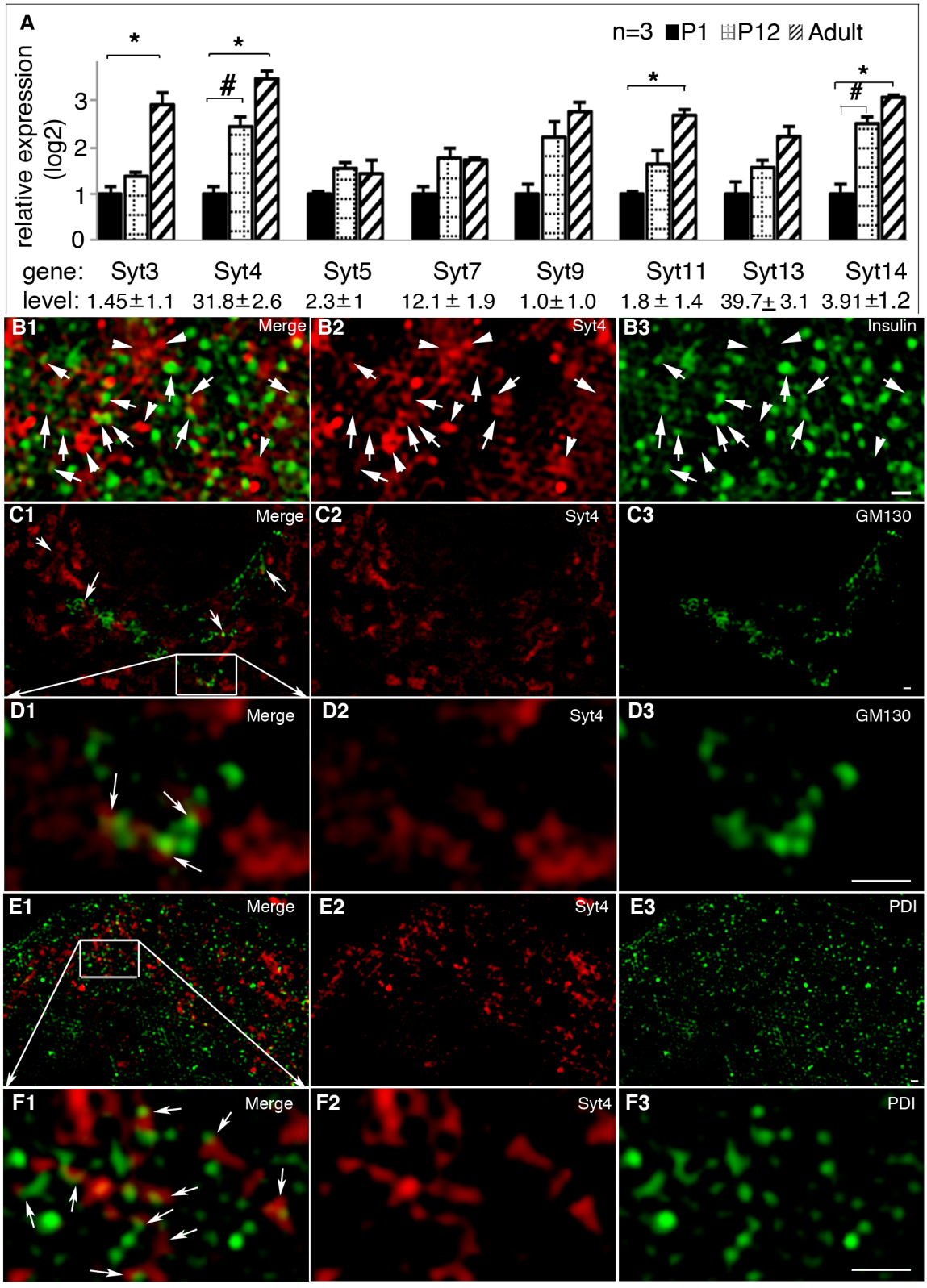
The oligo sequences are listed from 5’ to 3’ end. The expected sizes of DNA fragments for genotyping were provided.

Figure S1. Insulin granular size and cAMP signaling cannot account for the differential insulin secretion of immature and mature β cells, related to Figure 1.



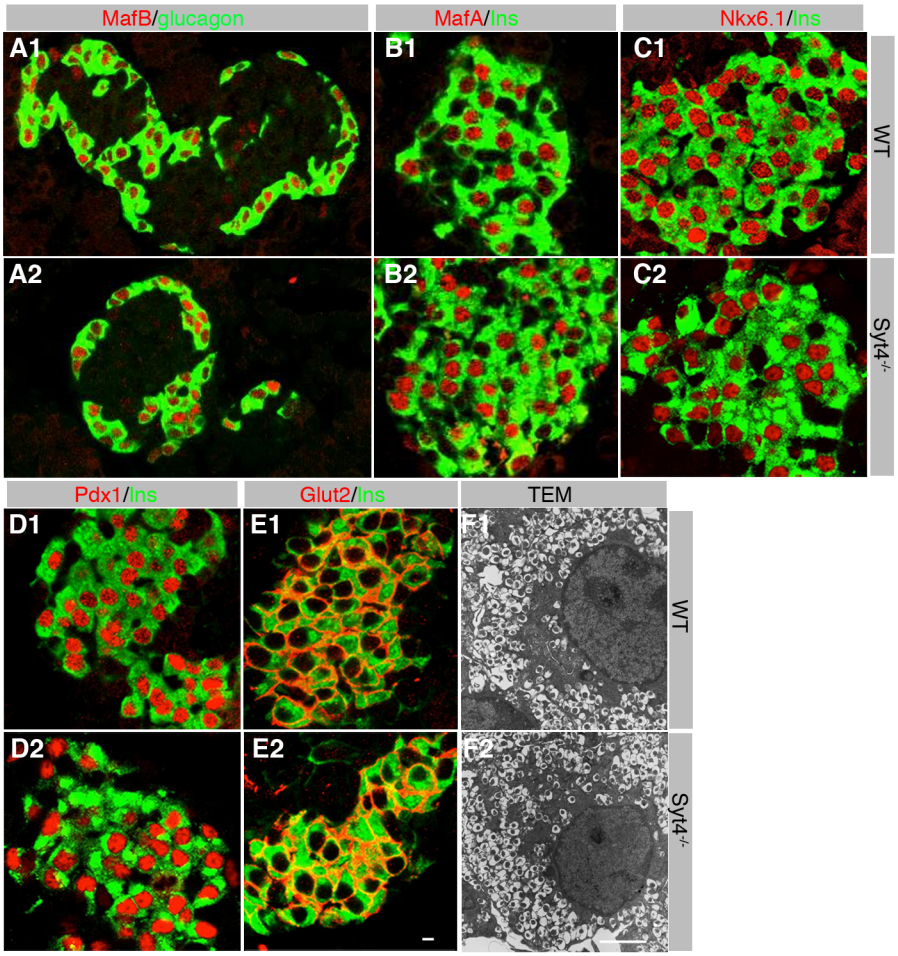
(A-D) TEM images of P1, P4, P12, and P60 ICR β cells. Arrows point to examples of immature vesicles. Arrowheads point to mature vesicles. (E) The diameters of insulin crystals in mature vesicles at different stages (n≥ 35). (F) Insulin secretion in response to KCl in P1 and adult islets with or without IBMX. The left columns are the % of insulin released within a 45 minutes window. The right columns are the fold of increase in insulin secretion induced by IBMX in P1 and adult islet. DMSO was used as control for IBMX. *: P<0.01.

Figure S2. Mature β cells have higher levels of Syt4, which associates with Golgi and ER, related to Figure 2.



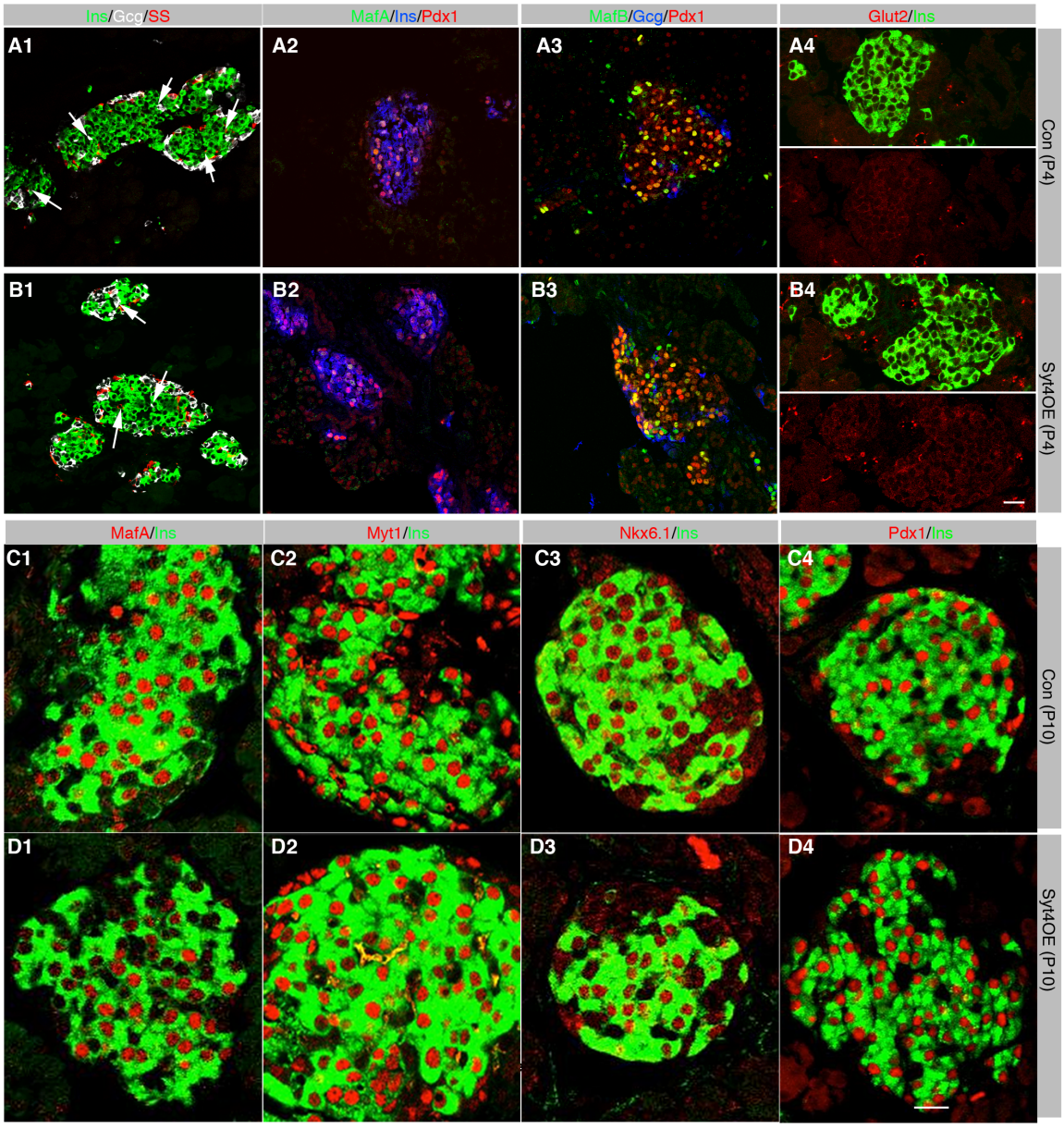
(A) Real-time RT-PCR assays of *Syts* mRNA in P1, P12 and adult β cells from *Rip*^{mCherry} mice. The relative gene levels are from P60 β cells, calculated based on real time RT-PCR assays (the *Syt9* expression was arbitrarily defined as 1). (*: P≤ 0.02, Between Subjects ANOVA tests. #: P<0.04, T-test). (B) Typical localization of Syt4 and insulin under SIM. In B1-B3, arrows point to insulin vesicles that have close association/overlapping with Syt4 signal; arrowheads point at Syt4 signal patches without insulin signals close by. (C, D) Localization of Syt4 in the trans-Golgi region, recognized by GM130. Panel D showed the boxed region in C. (E, F) Localization of Syt4 in ER, recognized by PDI. Single SIM slice was shown for panels B-F. Panel F showed the boxed region in E. Scale bars=3 μm.

Figure S3. Gene expression and vesicle morphology in *Syt4*$^{-/-}$ mutant islets, related to Figure 2.



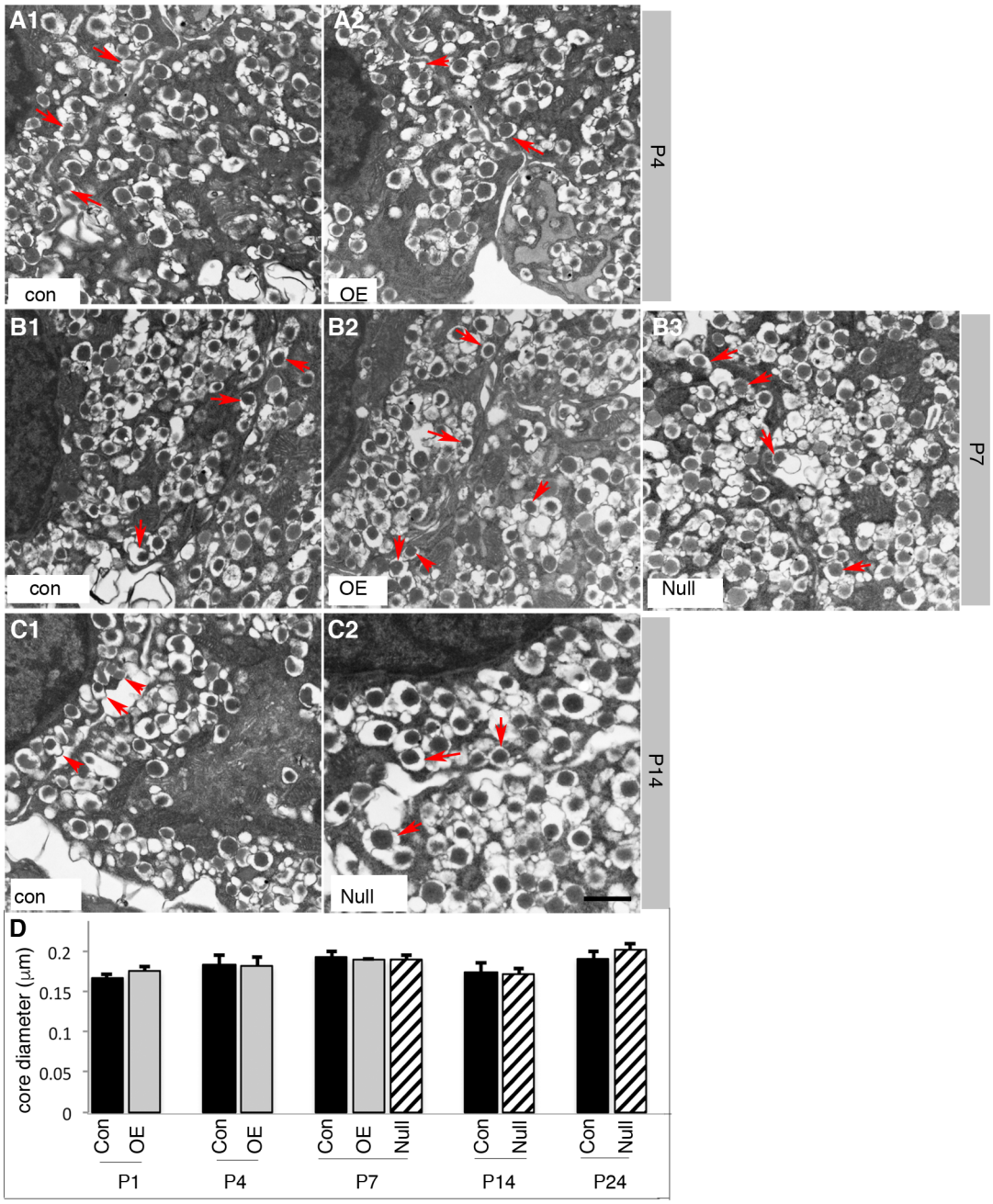
P10 pancreatic tissues (paraffin sections) were utilized. Presented are confocal images, with single optical slice. Shown molecules and genotypes were marked in each panel. Bars=2 μm.

Figure S4. Gene expression in *Syt4*^{OE} islets, related to Figure 3.



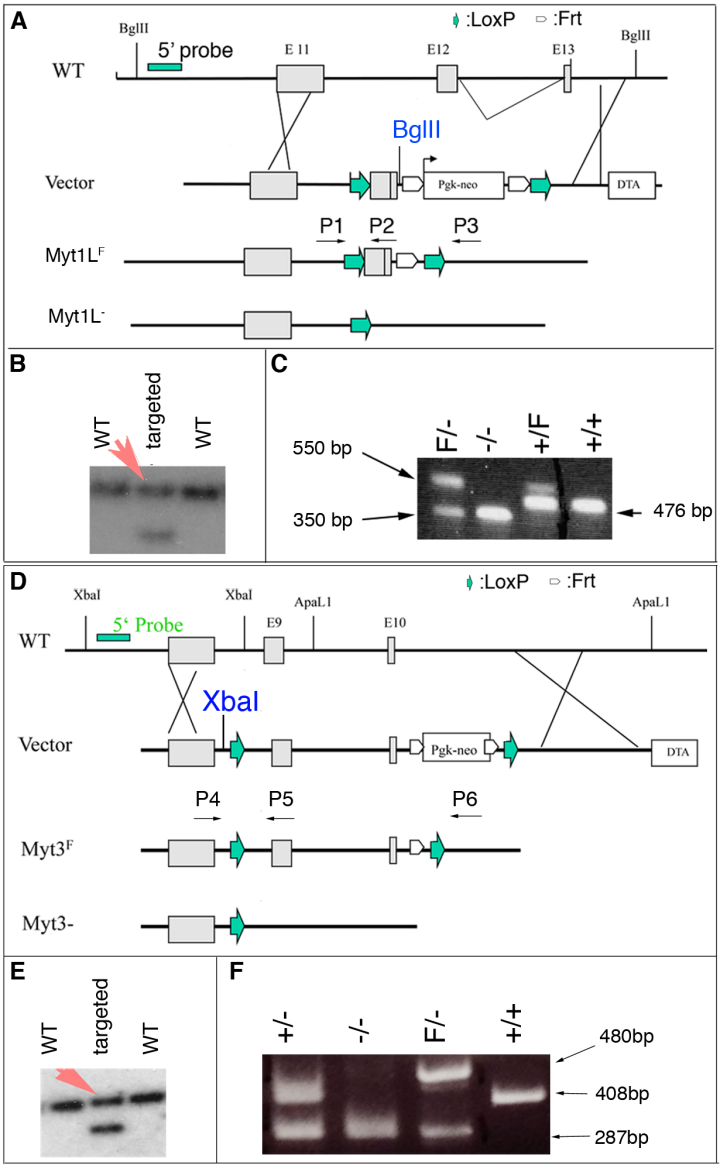
P4 and P10 pancreatic tissues (both cryo-sections and paraffin sections) were utilized. Presented are confocal images, with single optical slice. Tissues were processed and stained in parallel and images captured under identical parameters. Shown molecules and genotypes were marked in each panel. (A, B) Tissue staining results in P4 pancreata. (C, D) Tissue staining results in P10 pancreata. Bar=20 μm.

Figure S5. TEM characterization of docked insulin vesicles in islets of gain- and loss-of *Syt4* function mice, related to Figure 5.



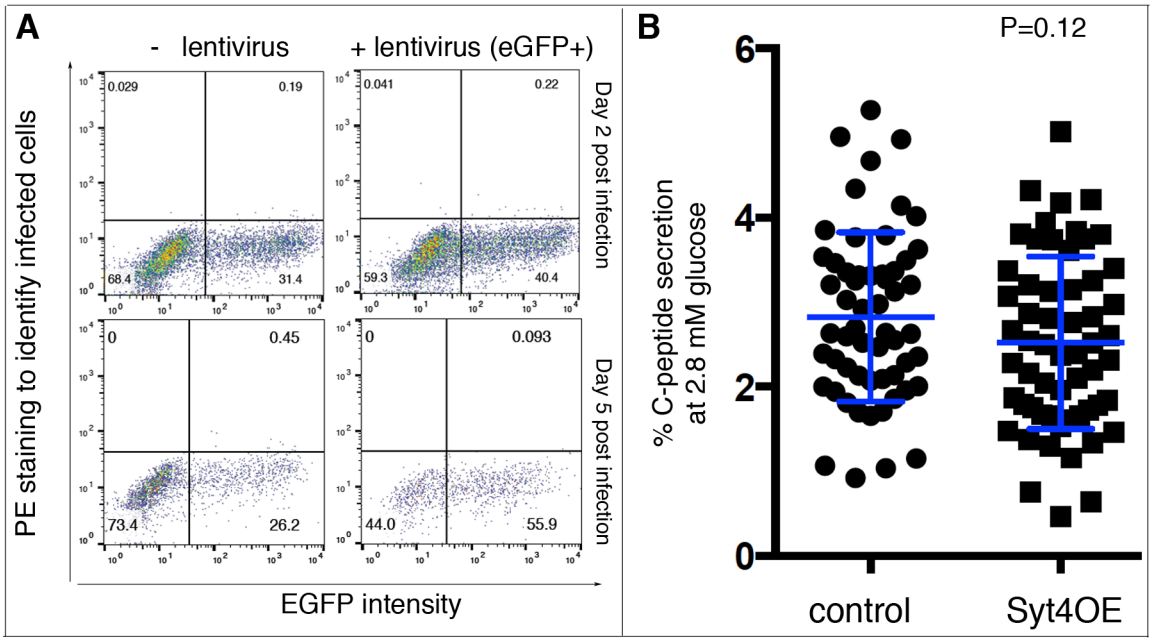
Representative TEM images of β cells at P4 (A), P7 (B), and P14 (C). At P4, control (con, A1) and *Syt4*^{OE} (OE, A2) β cells were shown. At P7, control (B1), *Syt4*^{OE} (B2), and *Syt4*$^{-/-}$ null (B3) β cells were shown. At P14, control (C1) and *Syt4*$^{-/-}$ (C2) β cells were shown. (D) Quantification of insulin dense core diameter at different stages. Bar=1 μm. Arrows point at some examples of docked vesicles.

Figure S6. Gene targeting strategies for the derivation of conditional *Myt1L*^{F} and *St18*^{F}, related to Figure 6.



(A) The making of *Myt1L*^{F}. The targeting vector contains, from 5' to the 3'end, 7.2 kb 5' arm, LoxP, fused exons 12 and 13, FRT-flanked pGKneo selection cassette, LoxP, and 2.5 kb 3' arm, and a DTA negative selection marker. A BglII site was introduced in the construct for later southern blot-based clone selection. (B) Southern blot with a 5' probe to identify an ES cell clone (pink arrow) with proper homologous recombination. BglII-digested genomic DNA of the targeted clone will yield a 8 kb band, compared with wild type clones that only have ~13kb bands. (C) PCR-based genotyping, with primers P1+P2+P3 indicated in (A). See SITable S2 for the sequences of oligos. (D-F) The derivation and genotyping of *St18*^{F} allele, following similar scheme as *Myt1L*^{F}, except southern blot used Xba I digestion.

Figure S7. Basal insulin secretion in human ES cell-derived β cells, related to Figure 7.



(A) Estimation of % of infected human β-like cells (with a virus carrying an *eGFP* reporter). The human ES cells carry a transgene that express *eGFP* under the control of an insulin promoter. Thus a third of cells were eGFP$^+$ without infection (two right images, lentivirus), indicating insulin transcription. Lentiviral infection increased the % of green cells (two left images, +lentivirus), indicating successful transfection. (B) The combined data from 5 independent experiments, showing the % of insulin released at 2.8 mM glucose within a 45-minute time window. P=0.12 (T-test).